

Single-scan swept source Mueller microscopy

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Introduction

Polarized light microscope¹ is widely used to observe biological structures² that present by themselves optical anisotropic properties such as refraction (birefringence) and absorption (diattenuation). Among polarization sensitive specimens, collagen fibrils, stress fibers made of filamentous actin and myosin, and microtubules display birefringence, while dye molecules associated to anisotropic transition moments exhibit diattenuation. Light polarization can also be modified randomly (depolarization) by scattering through specimens. In most configurations polarized light microscopes, made of two crossed linear polarizers, provide qualitative images by simplifying the specimen as a unique linear birefringence.

Materials and Methods

A full Mueller polarimetric microscope based on a wavelength-swept-laser source is implemented in a commercial laser-scanning microscope². The device uses no moving parts for the polarized state generator (PSG) and the polarized state analyser (PSA), but linear polarisers and fixed thick retarders to encode light polarization in spectral domain³⁻⁵. From the channelled spectrum measured by a single detector, the full Mueller matrix is determined at each point of the specimen according to the speed of the swept-source, i.e. 10 µs in our case. This new microscope is a promising tool due to (1) the simplicity of using passive PSG and PSA blocks, (2) the fast acquisition to image Mueller elements (0.65 s for a 256x256 pixel image), (3) the good signal-to-noise ratio by means of coherent illumination, (4) the possibility of combining nonlinear optic imaging modalities such as Second Harmonic Generation (SHG) microscopy.

Results and Discussion

We present a completed Mueller microscope provided with a real-time image module to display the change of polarization by the specimen and make the adjustment of the microscope easier. Another functionality has been added to switch from the Mueller microscope to a SHG microscopy configuration in order to image the same region of interest with the same objective and take advantage of the specific SHG signal sensitive to intrinsic nonlinear signatures of biological molecules such as collagen type II fibers. The performances of our Mueller microscope are illustrated by imaging several structural organisations of free labelled biological samples that can not be reduced to a simple linear birefringence. In particular all polarimetric parameters are imaged at a unique frame rate of 1.5 Hz, and unstained liver collagen fibers embedded in paraffin is revealed through a degree of alignment based on the structural organisation of fibers.

Bibliography

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